

Comparative Placental Morphology and Function

by Felix Beck*

The distinction between histiotrophic nutrition (in which local macromolecules are chiefly responsible for the maintenance of the embryo) and hemotrophic nutrition (which results from a transfer of material between the maternal and fetal circulations) is made. Placentation in a number of commonly used laboratory animals and in man is described, and it is shown that dependence upon histiotroph and hemotroph varies greatly, not only between species but also at different stages of gestation in a single species. These facts are likely to be reflected in considerable differences in response to certain teratogens; they must be carefully considered when experimental results are extrapolated between species. The significance to man of an agent which has been shown to be teratogenic in a single species of experimental animals should be evaluated in terms of possible differences in placental function between man and that species. This is particularly so if there is a suspicion that the potential teratogen may affect the fetal membranes.

Introduction

A discussion of comparative placental morphology and function is conveniently introduced by classification of the extraembryonic membranes involved in placental formation. This is best done by studying the relevant tissues in aquatic oviparous animals and developed by reference to more complex forms ending with the placenta of various eutherian mammals. Using this taxonomic approach we are better able to appreciate the biological potential of different fetal membranes and their capacity for modification (in concert with maternal tissue) to produce the extraordinary range of placental forms seen in the Eutheria. There is obviously an evolutionary corollary to this subject but it is complex and outside my present terms of reference. From the material to be presented here no conclusions can be drawn on that matter.

The most primitive fetal membrane is the yolk sac (Fig. 1). Early cleavage in bony and cartilaginous fish results in a blastoderm perched on a

mass of yolk. Gradually the ectoderm, mesoderm, and underlying periblast extend from the blastoderm around the yolk mass and surround it in a vascularized trilaminar yolk sac. Embryonic nutrition is due to absorption of yolk by the periblast and transfer of nutrients to mesodermal blood vessels for transmission to the embryo. The requirements of terrestrial life lead to the development of further fetal membranes by reptiles (Fig. 2). The shell membranes are modified to prevent desiccation, but this and other factors inhibit gaseous interchange and excretion. A diverticulum of the hind-gut—the allantois—is formed. Its cavity is lined by endoderm, and it is covered by vascularized mesoderm. This large sac receives and stores fetal urine and lies in the extraembryonic coelom. The latter develops as a result of a split in the extraembryonic mesoderm which separates an outer chorion (ectodermal trophoblast lined by avascular mesoderm) from the endodermal yolk sac covered by vascularized mesoderm. Allantoic mesoderm fuses with chorionic mesoderm to form the chorio-allantois, and by this means the allantoic circulation becomes greatly concerned with gaseous interchange. As will be seen later the chorio-allantois forms the

*Department of Anatomy, University of Leicester, Leicester, U. K.

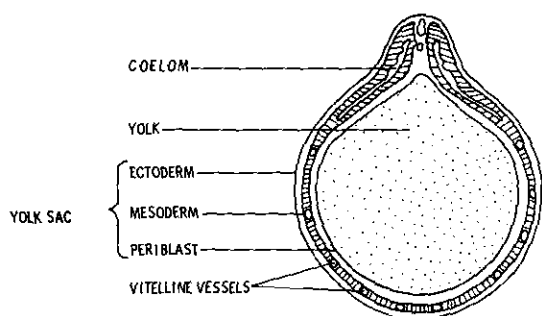


FIGURE 1. Diagrammatic section through an idealized large yolked anamniote conceptus.

basis of the mature eutherian placenta, and its function of gaseous interchange is thus greatly extended to embrace the passage of solutes between mother and fetus. In addition to all the above modifications, the reptilian embryo develops in an ectodermally lined fluid filled sac—the amniotic cavity (Fig. 2).

The development of viviparity confers great advantages upon the conceptus among which stability of environment is clearly the greatest. In truly viviparous forms, the embryo absorbs materials of maternal origin, and thus a reduction in the amount of egg yolk present occurs. This situation reaches its highest state of development in eutherian mammals. Newly born animals can thus have a far higher protein content than the fertilized ova.

Mossman's (1) definition of the placenta as an intimate apposition or fusion of fetal organs to maternal tissues for physiological interchange is still valid, and we must pause to consider which of the extraembryonic membranes are used for this purpose in Mammalia. In Metatheria it is principally the yolk sac, though some species do make restricted use of the chorio-allantois. Eutherian mammals, sooner or later during the course of gestation, use the chorio-allantois as their prin-

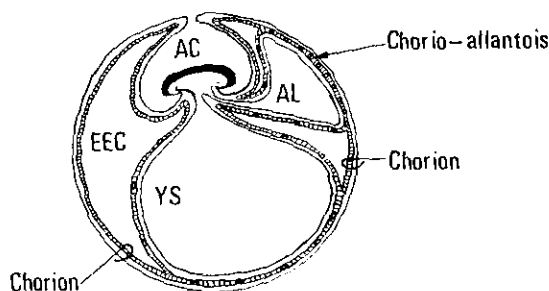


FIGURE 2. Diagrammatic section showing the extraembryonic membrane of an amniote: (AC) amniotic cavity; (YS) yolk sac; (EEC) extraembryonic coelom; (AL) Allantois.

cipal placental tissue, though it is often preceded and accompanied by some yolk sac-mediated nutrition; this arrangement is also seen in viviparous reptiles. It must be stressed however that (unlike viviparous reptiles) Eutheria possess a bewildering profusion of placental types, both topographically and histologically. Many structures other than the chorio-allantois and the unmodified yolk sac are used, and various laboratory mammals consequently differ greatly in placental function both as regards their histophysiology and the temporal sequence of events taking place at the placental site. In the mother, great differences in the local (endometrial) and systemic (mainly endocrine) response to pregnancy are also manifest and must naturally be considered in any attempt at a holistic view of the problems of placentation.

Embryonic Nutrition

A fundamental distinction should be made between histiotrophic and hemotrophic nutrition. Histiotrophic nutrition results from the intracellular breakdown of maternal macromolecules in the fetal membranes. These biopolymers are derived from endometrial cells, glandular secretion, and maternal blood or its transudate. Hemotrophic nutrition, by contrast, consists mainly of an interchange of solutes between maternal and fetal circulations across the so-called placental barrier (*vide infra*). While not wishing to state the obvious, it must be realized that hemotrophic nutrition cannot begin until a fetal circulation perfuses the extraembryonic membranes. At this stage organogenesis must be reasonably advanced, at least to the point of development of a beating embryonic heart. Space does not permit even a brief discussion of the factors which govern the passage of materials across the placental barrier (2). The majority pass by simple diffusion along a chemical or electrochemical gradient, some cross by facilitated diffusion and yet others by virtue of active transport mechanisms. Grosser's classification (3) which related the ease of passage of solutes between the maternal and fetal circulations to the number of cell layers between them is now considered merely a morphological convenience, since it does not reflect the thickness of the barrier or its cytophysiological characteristics. The Fick constant, functional surface area of the placental membrane concerned in the exchange process, size and charge of the solute under consideration, as well as its binding capacity to carriers on the fetal

and maternal sides are among the many factors which must be considered.

Histiotrophic nutrition usually involves the breakdown of maternal macromolecules in the vacuolar system of cells in the extraembryonic membranes. Many examples involving phagocytosis, pinocytosis, and micropinocytosis are available. The principle of heterosis involved is illustrated in Figure 3. Besides supplying the early nidus with its metabolic needs, heterosis is involved in the process of implantation and in many species continues to be an important factor in embryonic nutrition well after the full establishment of the chorio-allantois, often until full term. In special circumstances, such as the transfer of γ globulins from mother to young, whole macromolecules pass the placental barrier without the intervention of hydrolytic enzymes (4).

Comparative Placental Morphology and Function in Laboratory Animals: Correlation With Embryonic Development

The Rat

Implantation in the rat occurs at 5.5 days, 3 days before the beginning of gastrulation. Trophoblast penetrates the uterine epithelium

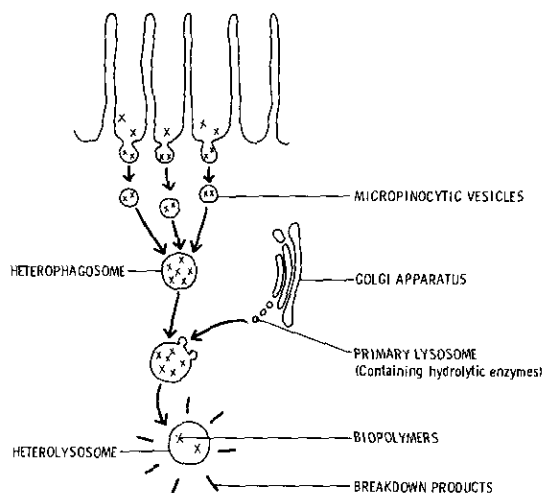


FIGURE 3. Diagram to illustrate the process of heterosis in its simplest form. There is evidence to indicate that heterophagosomes obtain hydrolytic enzymes by fusion with secondary lysosomes (e.g., heterolysosomes) as well as with primary lysosomes (this is not shown in the diagram). The process illustrated here is of micropinocytosis, but the uptake of macromolecules may equally well involve gross pinocytosis or even the uptake of solid particles, e.g., red blood corpuscles and endometrial cell detritus.

and actively invades the decidualized uterine stroma as well as the walls of the maternal capillaries. Some histiotroph is therefore taken up at this stage even though little apparent embryonic development occurs. Gastrulation begins at 8.5 days (Fig. 4c), at which stage a process known as entypy (inversion) of the yolk sac has developed because of growth of an ectoplacental cone composed of extraembryonic ectoderm. At this stage nutrition of the embryonic disc is probably largely due to the ability of the extraembryonic endoderm to degrade macromolecules in its vacuolar system. The histiotroph appears to come from three sources; the maternal serum located in lacunae between trophoblastic cells, endometrial cells broken down by trophoblast, and secretions of the uterine glands.

As pregnancy advances, the uterine cavity in the region of the implanted blastocyst is obliterated to reappear some days later on the antimesometrial aspect of the implantation site. After the appearance of the embryonic head, tail and lateral folds (at about 20 somites) the labyrinthine chorio-allantoic placenta develops as

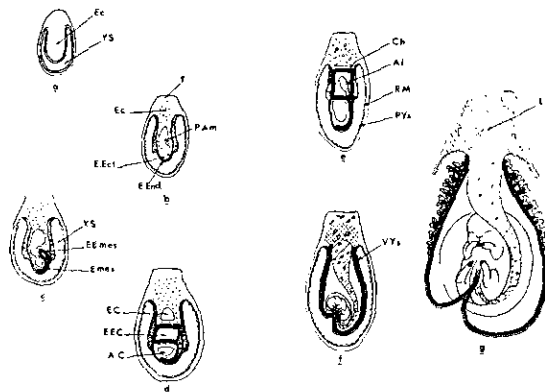


FIGURE 4. Diagram to show the disposition of the extraembryonic membranes in the rat: (a) at 6½ days (post implantation); (b) at 8 days (formation of pro-amniotic cavity); (c) at 9¼ days (gastrulation and early formation of extraembryonic mesoderm and coelom); (d) at 9½ days (division of egg cylinder into three cavities); (e) at 10 days (development of the allantoic bud); (f) at 11½ days (early chorio-allantoic placenta); (g) at 16 days—full term (disappearance of parietal yolk sac and Reichert's membrane). Inversion of the yolk sac occurs, and on day 16 the parietal wall of the yolk sac degenerates with the overlying trophoblast. The visceral yolk sac wall is then exposed to the uterine lumen: (AC) amniotic cavity; (Al) allantois; (Ch) chorion; (EC) epamniotic cavity; (EEC) extraembryonic coelom; (EE Mes) extraembryonic mesoderm; (Ec) ectoplacental cone; (EEct) embryonic ectoderm; (EEnd) embryonic endoderm; (EMes) embryonic mesoderm; (L) labyrinth; (PAm) proamniotic cavity; (PYS) parietal yolk sac; (R.M) Reichert's membrane; (T) trophoblastic giant cells; (VYS) visceral yolk sac endoderm; (YS) yolk sac cavity.

the result of fusion of the allantoic bud with the chorion, and a hemochorial relationship is established (Fig. 4f). Hemotrophic nutrition in the rat therefore only begins to be established at about 11 days of gestation when (for example) the forelimb buds are already in evidence. The yolk sac endoderm, however, continues to be active until full term. On day 16 of pregnancy its parietal layer together with the covering trophoblast disappears, and the visceral layer is exposed to the uterine cavity (Fig. 4g), where it is in an excellent position to pinocytize and degrade the uterine secretions.

Histochemical and biochemical evidence in support of the proposition that the yolk sac ingests and degrades horseradish peroxidase *in vivo* is available (5) and conclusive evidence that the 17.5 day organ digests ^{125}I -labeled bovine serum albumin has been obtained by Williams et al. (6). Subsequently Williams et al. (7) have been able to measure the rate of uptake (expressed as the so-called endocytic index) and breakdown of ^{125}I albumin *in vitro* (Fig. 5) and Moore et al. (8) have shown that the so-called endocytic index for this protein depends greatly on its degree of denaturation. Besides acting as an organ of intracellular digestion the rat yolk sac is responsible for the transfer of some γ globulin antibodies from mother to fetus, and there is also evidence that certain ions reach the fetus through the yolk sac rather than via the chorio-allantois.

The post-nidation rat embryo thus appears to pass through three phases of placental transfer.

An initial quiescent phase before gastrulation takes place during implantation by trophoblastic invasion; at this stage, decidual formation is pronounced, and maternal blood lakes form between some of the trophoblastic cells. The second phase, that of yolk sac nutrition, is present at the onset of gastrulation and is typified by great development of the visceral layer of the inverted yolk sac. The prime importance of that organ appears to be macromolecular breakdown of material for embryonic nutrition. Finally the chorio-allantoic placenta appears (at the limb bud stage) and takes over much of the nutritional function of the yolk sac; it is concerned with hemotrophic rather than histiotrophic processes.

Other Rodents and the Rabbit

Histricomorph rodents as exemplified by the guinea pig have a placental system very similar to that of the rat (a myomorph rodent). Implantation commences at 6 days of gestation and precedes gastrulation by 7 to 8 days. During this time penetration of the endometrium occurs, but the future embryonic cells are relatively quiescent. The yolk sac is inverted, and its function as an organ of histiotrophic nutrition is similar to that in the myomorph; it differs only in that the parietal yolk sac endoderm and Reichert's membrane never develop and the antimesometrial trophoblast degenerates before gastrulation thus exposing the visceral yolk sac at an early developmental stage. The hemochorial chorioallantoic placenta appears at about the same point in embryogenesis as it does in the rat (about 20 somites). It differs in morphological detail from that of the rat (for example it has a well-developed subplacenta) but the significance of the differences is obscure, and functionally both chorio-allantoic systems are probably broadly similar.

Placentation in the rabbit has many features in common with rodents, but sufficient dissimilarities exist to make it possible that some of these account for observed differences in response to teratogens. Central implantation of a swollen blastocyst coincides with gastrulation. First attachment at about 7 days is mesometrial and occurs in crescentic areas at the sides of the embryonic disc. In this region, the uterine epithelial cells proliferate vigorously to form a symplasmal mass while the uterine glands become highly secretory. A nutritional pabulum is thus formed at the point of trophoblastic penetration which is absorbed within 10 days, while decidual cells continue to develop in the uterine stroma. Endoderm and vascularized mesoderm spread out from the

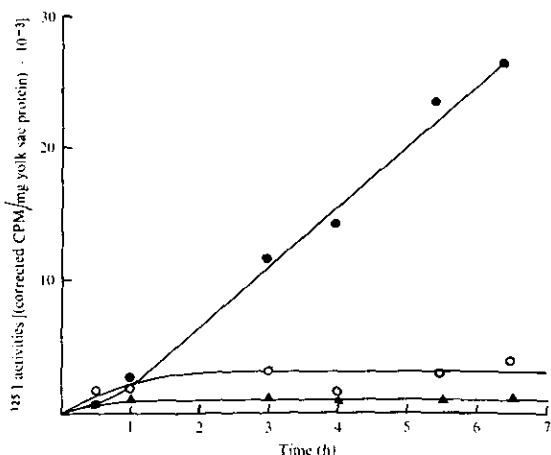


FIGURE 5. Levels of ^{125}I activity in tissue and culture medium on incubation of yolk sacs in the presence of ^{125}I Bovine serum albumin: (●) TCA-soluble activity released into the culture medium; (○) TCA-insoluble activity in the yolk sac; (▲) TCA-soluble activity in the yolk sac. Data at each time interval are from a single yolk sac. From Williams et al. (7).

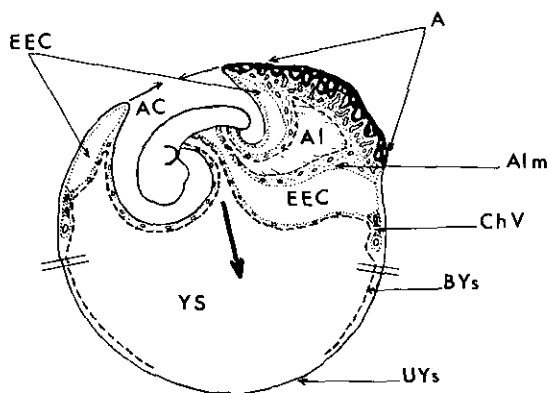


FIGURE 6. Composite diagram showing placental development in the rabbit: (A) chorio-allantoic placenta; (Ac) amniotic cavity; (Al) allantois; (Alm) allantoic mesoderm; (BYs) bilaminar yolk sac; (ChV) choriovitelline placenta; (EEC) extra-embryonic coelom; (UYs) unilaminar yolk sac; (YS) yolk sac cavity. The region of the chorio-allantoic placenta (A) is depicted at a later stage of development than the yolk sac. By this stage the amniotic cavity will have closed by fusion (arrows), the yolk sac will have become inverted by inward displacement of the embryo (thick arrow), the choriovitelline placenta will be completely devascularised and the yolk sac distal to the lines (||) will have disappeared.

embryonic area after implantation (the mesoderm extending only about halfway around the blastocyst to the sinus terminalis) and the overlying trophoblast thickens and penetrates the endometrium. An uninverted trilaminar yolk sac placenta (choriovitelline placenta) is thus formed in this region which is probably capable of both histiotrophic and hemotrophic nutrition. The extraembryonic coelom then splits most of the trilaminar yolk sac into an avascular chorion and vascular yolk sac which latter eventually becomes inverted with growth of the embryo and extra-embryonic coelom (Fig. 6). Below the sinus terminalis the bilaminar (trophoblast and endoderm only) and unilaminar yolk sac undertakes histiotrophic nutrition as the trophoblast penetrates a new area of uterine epithelium which has undergone symplasmal transformation. Eventually (about 15 days) the bilaminar yolk sac degenerates and a true inverted yolk sac nutritional system similar to that found in rodents is established, the last portion of the choriovitelline placenta being devascularized at about the branchial arch stage of development. Chorio-allantoic placental development is established mesometrially at about the 20-somite stage when the forelimb buds have appeared (cf. rat).

In distinction to rodents, there is therefore no quiescent phase of embryonic development between implantation and gastrulation. This may be

reflected in the copious source of histiotroph provided by the uterine symplasma and the development of a choriovitelline placental system. The inverted yolk sac system does not begin to be important until just before development of the chorio-allantois.

The Ferret

This convenient laboratory animal has a placental system typical of mustelid carnivores. Central implantation at 12 days is coincident with gastrulation (cf. rabbit). As in the rabbit, the endometrial epithelium forms symplasmal masses which are penetrated by tongues of trophoblast responsible for histiotrophic nutrition. This is supplemented by absorption of uterine secretions from the so-called mesometrial gutter (Fig. 7a). The choriovitelline placenta developed at this stage is probably mainly histiotrophic in function because there is no vascularised mesoderm in the cores of the invading trophoblastic villi. Closure of the amnion is followed by extravasation of maternal blood into the antimesometrial aspect of the implantation chamber where the red blood corpuscles are absorbed by trophoblastic cells in the so-called hemophagous organ (Fig. 7b) presumably to provide a source of iron for the developing embryo. With growth of the allantois into a rapidly expanding extraembryonic coelom the chorioallantoic placenta develops (Fig. 7b) and eventually replaces the choriovitelline form at about 20 days when the embryo has 35 or more somites. Chorio-allantoic placental development is somewhat different from the hemochorial pattern of rodents and rabbits;

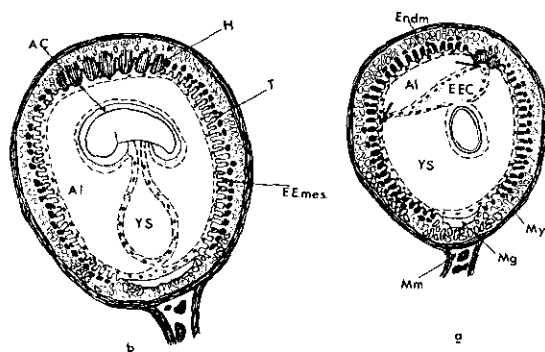


FIGURE 7. Extraembryonic membranes of the ferret (a) at 19 days and (b) at 21 days: (AC) amniotic cavity; (AI) allantois; (EEC) extraembryonic coelom; (EEmes) extraembryonic mesoderm; (Endm) endometrium; (H) hemophagous organ; (Mg) mesometrial gutter; (Mm) mesometrium; (My) myometrium; (T) trophoblast; (YS) yolk sac cavity.

the placental barrier is thick and an endothelio-chorial relationship between the maternal and fetal circulation is established (9, 10). Histiotrophic nutrition persists in the paraplacental chorion and mesometrial gutter until term.

The Pig

Though this species is somewhat uncommon in teratological work, brief reference to the Ungulate placenta should be made (Fig. 8). The chorio-allantois is epithelio chorial in type but areas exist in which the placental barrier is thin and true hemotrophic nutrition occurs side by side with regions in which histiotrophic digestion of endometrial secretions is preponderant. Gastrulation and implantation (in this case intimate contact between trophoblast and endometrial epithelium) coincide, and a histiotrophic bilaminar yolk sac is responsible for early nutrition, to be replaced by a choriovitelline form which is still probably largely histiotrophic in function. The chorio-allantoic placenta begins to function at the limb bud stage but is maximally effective as a hemotrophic organ much later than in the forms previously described.

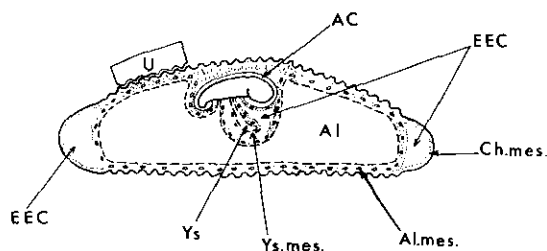


FIGURE 8. Extraembryonic membranes in the pig: (AC) amniotic cavity; (Al) allantois; (AlMes) allantoic mesoderm; (ChMes) chorionic mesoderm; (EEC) extraembryonic coelom; (U) uterine wall (endometrium and myometrium); (Ys) yolk sac cavity; (YsMes) yolk sac mesoderm after Mossman (1).

The Human

The human placental system is so well documented that only a brief summary need be given here. Implantation probably begins at about 7½ days, and a small plaque of trophoblast is formed at the point of penetration. Histiotrophic nutrition commences by digestion of a pabulum of endometrial tissue and secretion. Within 4-5 days the blastocyst becomes completely buried in the stratum compactum, and lacunae, first filled by endometrial secretion and later with maternal blood, develop between the cells of the syncytiotrophoblast.

A sluggish circulation develops at about 13 days of gestation and immediately the chorionic vesicle enlarges with the formation of an extraembryonic coelom (Fig. 9). Within 3 days gastrulation begins, and although nutrition is still histiotrophic it occurs largely from the maternal blood in the trophoblastic lacunae. Less than a week after the beginning of gastrulation an active circulation is established in the chorionic villi and embryonic nutrition becomes, at least to a considerable extent, hemotrophic. The characteristic features of human placentation therefore are a relatively quiescent phase of embryonic development during which interstitial implantation occurs and nutrition is histiotrophic; the chorionic vesicle begins to grow rapidly as soon as a maternal circulation begins to develop in the trophoblastic lacunae, and this is rapidly followed by gastrulation. Early embryonic development is characterized by precocious formation of the circulatory system with the result that perfusion of the chorio-allantois establishes hemotrophic nutrition (in a hemochorial placenta) at about 5 somites, i.e., much more quickly than in other mammals. Placental differentiation after formation of tertiary villi is concerned with regional specialization and does not change the basic mode of placental function, except insofar as there is a gradual thinning of the placental barrier as gestation proceeds. Some degree of histiotrophic nutrition however (mediated, for example, by the cytotrophoblastic shell) probably exists throughout gestation and certainly well into the second half of pregnancy.

Other Higher Primates

Old World monkeys such as the macaque have superficial implantation with a rather less in-

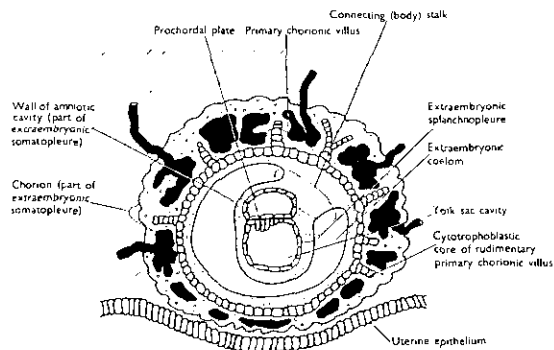


FIGURE 9. Diagram of the human chorionic vesicle at the stage of formation of the extraembryonic coelom. The maternal blood lacunae pervading the syncytiotrophoblast are shown in black. From Beck, Moffat, and Lloyd (11).

vasive trophoblast than that found in man. This becomes attached to a well marked epithelial plaque of endometrium which is absent in the human and no doubt is a potent source of histiotroph. As in man vascular maternal lacunae form between the trophoblastic cells and in the macaque chorionic villi grow into the lacunae. Within two days gastrulation occurs and once again the cardiovascular system develops precociously so that hemotrophic nutrition (i.e., the development of tertiary placental villi) is established at about 5 somites—only a little after similar events occur in man.

In New World monkeys such as the marmoset, superficial implantation is afforded by an extensive proliferation of trophoblast involving a large area of the blastocyst and a reticulated syncytiotrophoblast penetrates the uterus. A large endometrial epithelial plaque and much glandular secretion show that histiotrophic nutrition is far better developed in these early stages than in rhesus or man. Eventually, cytotrophoblastic villi with a mesodermal core penetrate into the reticulated syncytiotrophoblast and replace the latter by a placenta that looks very like that of the Old World monkey. However, the whole has taken far longer to develop than the Old World monkey placenta and the marmoset is clearly dependent upon histiotrophic nutrition for a far longer period than is the macaque.

Both Old and New World monkeys possess decidual cells which are smaller than those in the human. Furthermore, the gross form of the placenta is often double discoidal, but this is of doubtful consequence in functional terms.

Conclusions

This presentation has described the placental system in some laboratory animals in order to see whether they make appropriate models for comparison with man. Clearly the simian primates most nearly approximate to the ideal but important differences between them and man still exist. Even in Old World monkeys, but particularly in the New World species, histiotrophic nutrition is more important in early development than it is in the human. This is quite likely to affect the way in which drugs and other potential teratogens are handled at these critical developmental stages.

In the rat, which is the cheapest and most widely used test species, a true hemochorial chorio-allantoic placenta is not established until about the 20-25 somite (anterior limb bud) stage. Before that, histiotroph forms the only source of

nutrition. A further complication is that during the greater part of organogenesis histiotrophic nutrition occurs by a breakdown of a transudate of maternal serum in the endoderm of the inverted yolk sac placenta. The capacity of the latter to endocytize macromolecules has been shown to be affected by circulating teratogens (such as trypan blue) which cease to be effective as soon as the chorio-allantoic placenta is established (12).

Though inverted yolk sac nutrition begins rather later in lagomorphs, its characteristics appear to be similar to that of rodents. In carnivores, as typified by the ferret, histiotrophic nutrition in one form or another seems to be the major source of nutrition until day 19-20 of gestation, when about 35 somites are present in the embryo and the limb buds are already well developed. The situation is also complicated by the fact that the chorio-allantoic placenta is endothelio-chorial in form and the placental barrier is thicker than in all other mammals. This contrasts

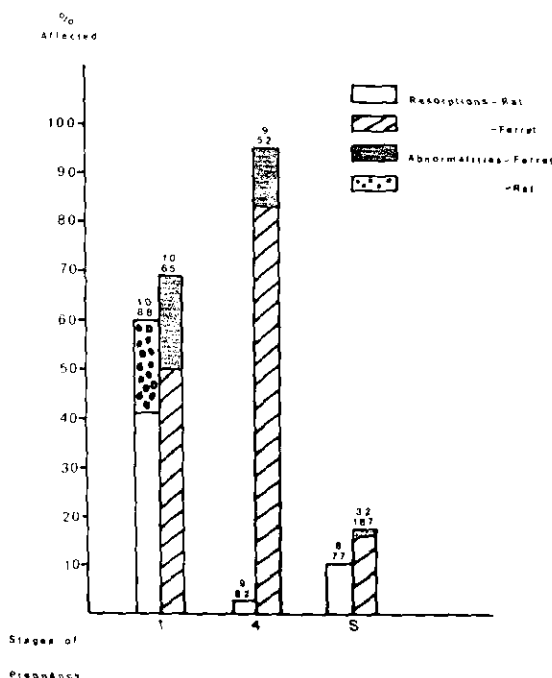


FIGURE 10. Externally visible abnormalities in fetuses following injection of trypan blue (50 mg/kg into ferrets and 100 mg/kg into rats) at (1) the primitive streak and (4) 23-33 somite stages of development in the rat and the ferret. The number of implantation sites is shown at the top of each block and the number of mothers used above this. Saline-treated controls are marked S. Both species respond to trypan blue when histiotrophic nutrition is the only form available (stage 1). At stage 4, the rat has a functional hemochorial placenta, while in the ferret morphological evidence indicates that nutrition is still mainly histiotrophic.

with the attenuated barrier in the hemochorial placentae present not only in simian primates but also in rodents and rabbits. In this connection, it is interesting to note that the response of ferrets to the teratogenic action of trypan blue is qualitatively and quantitatively completely different from that of rats (Fig. 10).

Experimentalists who work with ungulates must recognize a prolonged period during which the embryo is entirely dependent upon histiotroph. In the pig the chorio-allantois probably functions as a hemotrophic organ only at the stage of digital ray formation, and even then histiotroph remains vitally important, probably until term. It is interesting, nevertheless, that although the ungulate placenta is epithelio-chorial, the placental barrier in certain regions is thinner than that found in carnivores.

In summary, no experimentally available placental model is identical to man. Therefore it is only by an intelligent understanding of any system used that a proper appreciation of the applicability of results obtained to the human situation can be made.

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